SUPPLEMENTARY MATERIALS

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. LC/MS/MS analyses of mouse epidermal sphingosines and sphinganines during skin development. Epidermal sphingosines and sphinganines were measured in the skin of **(a)** E16.5, **(b)** E17.5, **(c)** E18.5 embryos and **(d)** P0 wildtype and Ctip2$^{-/-}$ mice. The amount of skin sphingosines and sphinganines were determined according to their N-acyl chain length by LC/MS/MS analyses, quantified utilizing sphingosine and sphinganine standards, and expressed as pmol/ mg of skin. Statistical analyses were performed by student's unpaired *t*-test using Graphpad Prism software. Data were reported as mean ± SEM (n=9). The results represent three separate experiments performed in triplicate.

Figure S2. UPLC/MS/MS analyses of mouse epidermal cholesterol and fatty acids during skin development. Lipids were extracted from skin of E16.5, E17.5, E18.5 embryos and P0 wildtype and Ctip2$^{-/-}$ mouse epidermis. Amount of **(a)** cholesterol, **(b)** cholesterol 3-sulfate, **(c)** palmitic acid and **(d)** stearic acid were determined using UPLC/MS/MS analyses. Statistical analyses were performed by student's unpaired *t*-test using Graphpad Prism software. *$p<0.05$. Data were reported as mean ± SEM (n=9). The results represent three separate experiments performed in triplicate.

Figure S3. Relative expression of genes encoding lipid-metabolizing enzymes during skin development. Expression of ceramide synthases (*Lass4, Lass5*), serine palmitoyltransferase 2 (*Sptlc2*), dihydroceramide desaturase (*Degs1*), 3-ketodihydrosphingosine reductase (*Kdsr*) and sphingomyelin synthase 1 (*Sgms1*) was studied at **(a)** E16.5, **(b)** E17.5 and **(c)** E18.5 stages of development by qRT-PCR using specific primers indicated in table S1. Values represent relative transcript level after normalization with *Gapdh* transcripts. Statistical analyses were performed by student's unpaired *t*-test using Graphpad Prism software. Data were reported as mean ± SEM (n=9). The results represent three separate experiments performed in triplicate.

Figure S4. ChIP analyses on murine keratinocytes for lipid metabolizing genes. Chromatin immunoprecipitation (ChIP) assay was performed on freshly isolated primary keratinocytes from neonatal mouse skin using anti-Ctip2 antibody and results were analyzed by qPCR using specific primers indicated in table S2. Rat IgG was used as a control. Ctip2 was not recruited to distal or proximal (relative to transcription start site of each gene) promoter regions of **(a)** *Lass1,* **(b)** *Lass2*, **(c)** *Lass6*, **(d)** *Ugcg*, **(e)** *Smpd1*, **(f)** *Smpd2*, **(g)** *Smpd3,* **(h)** *Sgms2*, **(i)** *Elovl4,* **(j)** *Dgat2* and **(k)** *Aloxe12b*. Statistical analyses were performed by student's unpaired *t*-test using Graphpad Prism software. The results represent three separate experiments performed in triplicate.

Figure S5. Schematic representation of Ctip2 mediated differential regulation of genes encoding different enzymes of the ceramide synthesis

pathways. The scheme indicates metabolic pathways for ceramide biosynthesis: *de novo* pathway, salvage pathway, sphingomyelinase pathway and exogenous ceramide recycling pathway. Genes involved in these pathways are indicated. Stages at which altered gene expression was observed are indicated within parentheses. Small arrows indicate upregulation/downregulation of genes. Ctip2 positively regulates Gba2 and Lass2 expressions by direct recruitment to their promoter regions.

Expression of ceramide synthases 1-6 (*Lass 1-6*), serine palmitoyltransferases 1-3 (*Sptlc 1-3*), dihydroceramide desaturase (*Degs1*), 3-ketodihydrosphingosine reductase (*Kdsr*), glucosylceramide synthase (*Ugcg*), acid beta-glucosidase (*Gba2*), sphingomyelin synthases 1-2 (*Sgms1-2*), sphingomyeinases 1-2 (*Smpd1*, *Smpd2*), N-acylsphingosine amidohydrolases (acid ceramidase Asah1 and neutral ceramidase *Asah2*), alkaline ceramidases 1-2 (*Acer1, Acer2*), sphingosine kinases 1-2 (*Sphk1, Sphk2*) are indicated for the different pathways.

Supplementary table 1. Sequence of primers used in qRT-PCR.

Gene	Sense	Antisense
Gapdh	5’-AGGTCGGTGTGAACGGATTTG-3’	5’-TGTAGACCATGTAGTTGAGGTCA-3’
Lass1	5’-ATGCCACGTGGAGGAACTA-3’	5’-ATTCGACAGGTCAAAGACGA-3’
Lass2	5’-TGATGGCAGTGCTACAGATG-3’	5’-CCCCCTCTGAACTCTCTGTT-3’
Lass3	5’-CTTCCTCAACCTCCAACTCA-3’	5’-CCTCTTCCTCTTCCTCTTCG-3’
Lass4	5’-GCCCCTTCTTTGGCTACTAC-3’	5’-ATCGCTACGAATGTCCTCTG-3’
Lass5	5’-GCTCTTTCTCACCTCCTTCC-3’	5’-GAGGTTGTTTTTGTGGGTTG-3’
Lass6	5’-AAAAGGCAAGGTATCCAAGG-3’	5’-CAAGGACCAGTGAGGAGGTA-3’
Sgms1	5’-TCTGGTGGTATCACACGATG-3’	5’-GCCAATGGTAAGATCGAGGT-3’
Sgms2	5’-CTGGGATCATCTGCATTCTC-3’	5’-GTTCGTCTGGGAAGAGACCT-3’
Smpd1	5’-TCACGTGGATGAGTTTGAGA-3’	5’-CCGGGGTAGTTTCCATCTAT-3’
Smpd2	5’-CTCACAGTGACAAGCCCTTC-3’	5’-CCTTAGCACGCTGATCAAAT-3’
Ugcg	5’-TGCCTGGCATGGTTTATATT-3’	5’-TAATGCCGACAGGAAAATGT-3’
Gba2	5’-CACGAGTTTGCAGAGAAGAGG-3’	5’-ATTGAGCATGTCGATGAAGCC-3’
Sptlc1	5’-TGGACAAGGAAGAGAAGTGC-3’	5’-AGGAGCCTACAACAGCACAG-3’
Sptlc2	5’-CATTGAGTCCAGAGCCAGAT-3’	5’-AAAGGGCCTGTCCAGTAGAG-3’
Sptlc3	5’-CTACTTCCCTGCCAGAAGGT-3’	5’-TATAGCACGCCCTGATTTCT-3’
Degs1	5’-GAATGGGTCTACACGGACCAG-3’	5’-AGTCATGGAGTGGTTAAGGCA-3’
Kdsr	5’-CGGTGACTTCCATCACTGAA-3’	5’-CTTCAGGTTTTGCTTTCTGC-3’
Asah1	5’-TCCGTGGCACACCATAAATCT-3’	5’-TCCACTTGGCACAAATGTATTCA-3’
Asah2	5’-TTCTCACCCTCTTGTTTGTTACC-3’	5’-AGGGAAGTTTGGAGTCTGTGT-3’
Sphk1	5’-CACCAGAACGGAAGAACCAT-3’	5’-GGTTTCTGGATGGCAGTCTC-3’
Sphk2	5’-CTGCTTTACGAGGTGCTGAA-3’	5’-CAACAGGTCAACACCGACAA-3’
Acer1	5’-GCTGGATCAGTGACCGTGTA-3’	5’-GGCATCTCATACTTTGCATCC-3’
Acer2	5’-GTGCCCTAGTACACCCAGGA-3’	5’-CCTTTCGCCATCTGGTAGTC-3’
Acer3	5’-CCTGAGGTACAGGCCAAAAG-3’	5’-TCTTGACCATCTTGGCAGAA-3’

Supplementary table 2. Sequence of primers used in ChIP-qPCR assay.

Gene	Sense	Antisense
Lass1-distal	5'-GACAGGCAGAGATCAGTGGA-3'	5'-AGCACTGAGGACGATGTCTG-3'
Lass1-proximal	5'-GAGTACCAACAACCCAAGCA-3'	5'-GAAGGTGAGGACAGACTGACA-3
Lass2-distal	5'-GTAGTTCAGGGGCAGAGAGC-3'	5'-CCATGGCAAGCACTTTTCTA-3'
Lass2-proximal	5'-CTGCTGCTCTCTTCTTGAGG-3'	5'-GCCCTGCTGTTTAGAGTCCT-3'
Lass2-3'UTR	5'-CATTCAACCAGGGAAACATC-3'	5'-TGTATCAACCCACCTTTGCT-3'
Lass3-distal	5'-GTATGGATGTGAGGCATGTG-3'	5'-GCCAGAAGACGGTTACAGAA-3'
Lass3-proximal	5'-ACAGTGTTCCGAGTTGCTCT-3'	5'-GGTACACGGGCATTAGTCTG-3'
Lass4-proximal	5'-GGTCTGGAGGAGACAAACGA-3'	5'-CTTGGTCCAAAACCCTGAGT-3'
Lass4-distal	5'-GCCAATGAGATGGATCAGGT-3'	5'-GCAGTTTGCAAAGGTCAGTT-3'
Lass4-3'UTR	5'-TTGAGTGGCACCTTTTCTTG-3'	5'-CCATAGAGGAAATGGACTTAGC-3'
Lass6-proximal	5'-CTGGCGTTCTAACCAGGTG-3'	5'-GCTAGTGTTCCCGTCAAGC-3'
Lass6-distal	5'-GAGCCGGAACTCATTGATCT-3'	5'-CACACACACACACACACACG-3'
Ugcg-distal	5'-GAGAGCACCCAAACAGAGAA-3'	5'-CTGCCCCCAATCCTATACTT-3'
Ugcg-proximal	5'-AATTCAGCATCAAGCGATTC-3'	5'-TGCACTGTCTCTAAGCAGCA-3'
Gba2-distal	5'-CTGGGAGGAGTGAGATGTGA-3'	5'-CTCCTGGTTTTAGGGACCAT-3'
Gba2-proximal	5'-GCGTCTCCAAACTCAGATTC-3'	5'-CCTCAACCCTGAAATGTGAC-3'
Gba2-3'UTR	5'-TAGAAGTGCCAGGACTCTGC-3'	5'-CTCCTGGGTCTCTTTCCATT-3'
Smpd1-distal	5'-TCACCTTGTCTGAGGCTACG-3'	5'-GTCAGAGCTGTTCCAAGCAA-3'
Smpd1-proximal	5'-TCTACCGAGGTCACCTACCC-3'	5'-GAGCGGCTGTCAAAACCT-3'
Smpd2-distal	5'-AGCTGTCCTCAGACACTCCA-3'	5'-TCTTCCGAAGGTCAGGAGTT-3'
Smpd2-proximal	5'-CCACCACCTTCAGCTTCA-3'	5'-CTATTTGTGTTCCGGGCTCT-3'
Smpd3-distal	5'-ACTCGGAGTCAGACAAAAGG-3'	5'-ACAGAAGCCTAGCGAAGAAA-3'
Smpd3-proximal	5'-GACGCTAACCACAGATCACA-3'	5'-GTGGGAGGACTGTTTCCATA-3'
Sgms2-distal	5'-TGACACGCAAGTAAGCTTTG-3'	5'-TCAAACCTGACCAAGGAAAA-3'
Sgms2-proximal	5'-GTACTGGGCTCACAGAATGG-3'	5'-AATCACTCTTTTCCCCCAAG-3'
eLox3-distal	5'-CCTTCACTCCCTGCCTTT-3'	5'-CGTGCTGCCTAGATCCTTAT-3'
eLox3-proximal	5'-CAGGCAGGTCCTATCTCAAA-3'	5'-GACAAGGCCATCATGTTCTC-3'
eLox3-3'UTR	5'-CCTAACCCTCATTCCAGCTT-3'	5'-TCTGACCCCCATACATGAAC-3'
Elovl4-distal	5'-GCTGCAGTTATTGTTGTTGC-3'	5'-CCACATGAGATTCAGGTCAA-3'
Elovl4-proximal	5'-ATATTTACGGGGTGACCACA-3'	5'-CATGTGCTTTTGCTGTCTTC-3'
Dgat2-distal	5'-TGGGCTCTGTGCATAGTTTA-3'	5'-GCGCACTTTATAACCTCACA-3'
Dgat2-proximal	5'-CCACTCATCATGCAAGTGTT-3'	5'-TCTAGGTCAATAGGGCCTTG-3'
Aloxe12b-distal	5'-CTGTGAAGGGAAGGAACTGA-3'	5'-TAGGACCCAATGAGGACTGA-3'
Aloxe12b-proximal	5'-GGGGCGAACAGCTTATTAGT-3'	5'-AGAAGATGCTGAGGCTGAAG-3'

Note: Amplified region for distal and proximal promoter is relative to the transcription start site of indicated gene and amplified region for 3'UTR is relative to the transcription stop site of indicated gene

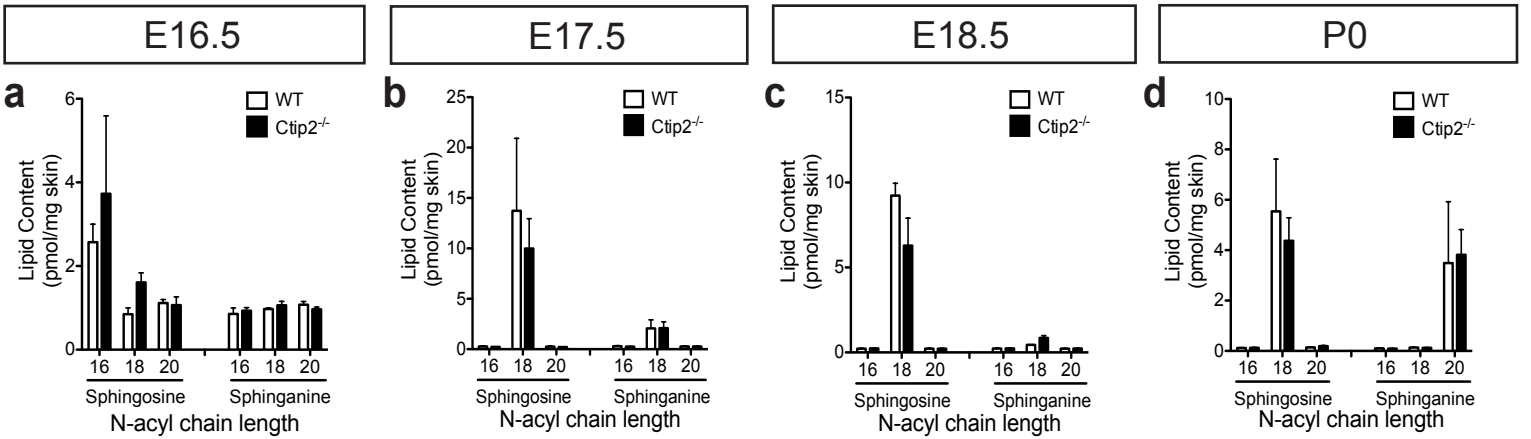

Figure S1, Wang et al., 2012

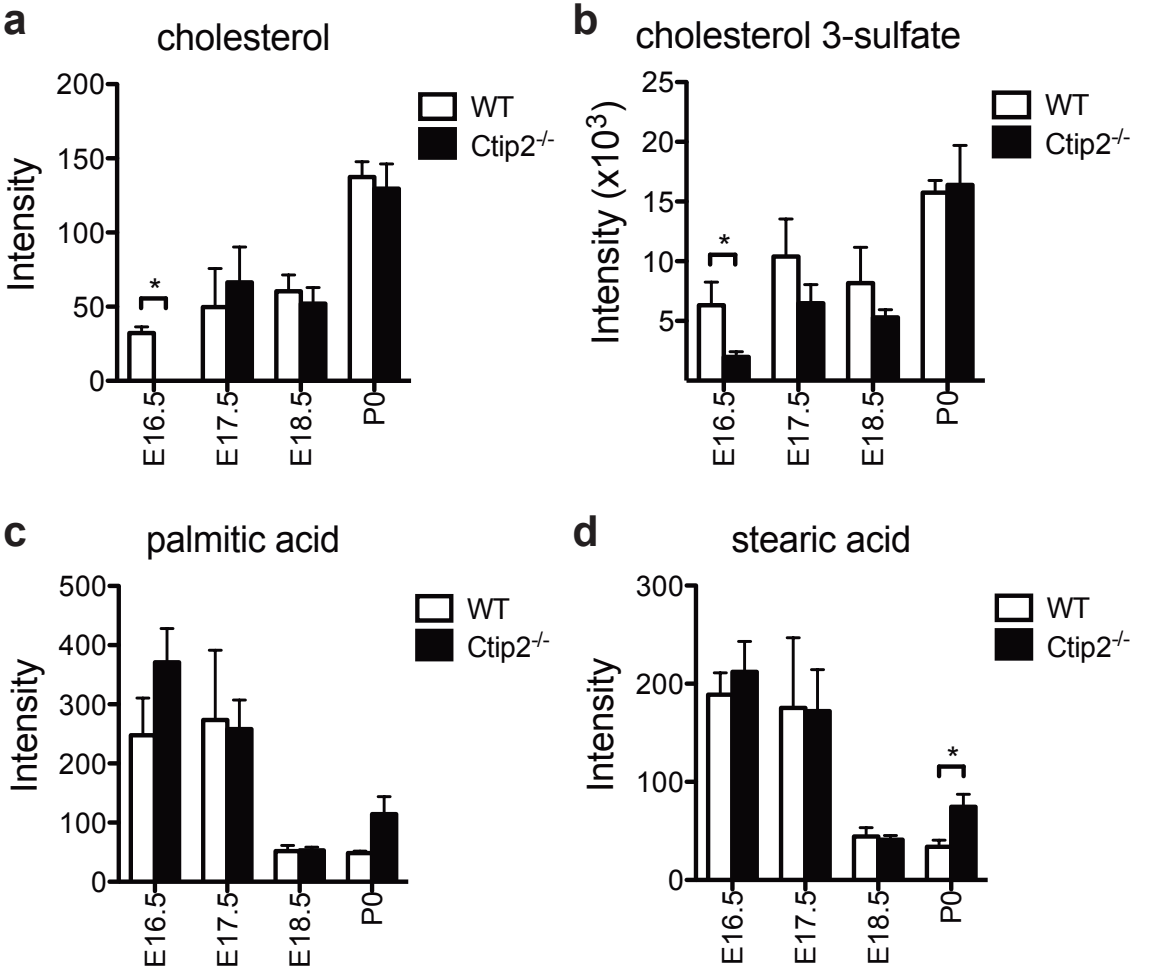

Figure S2, Wang et al., 2012

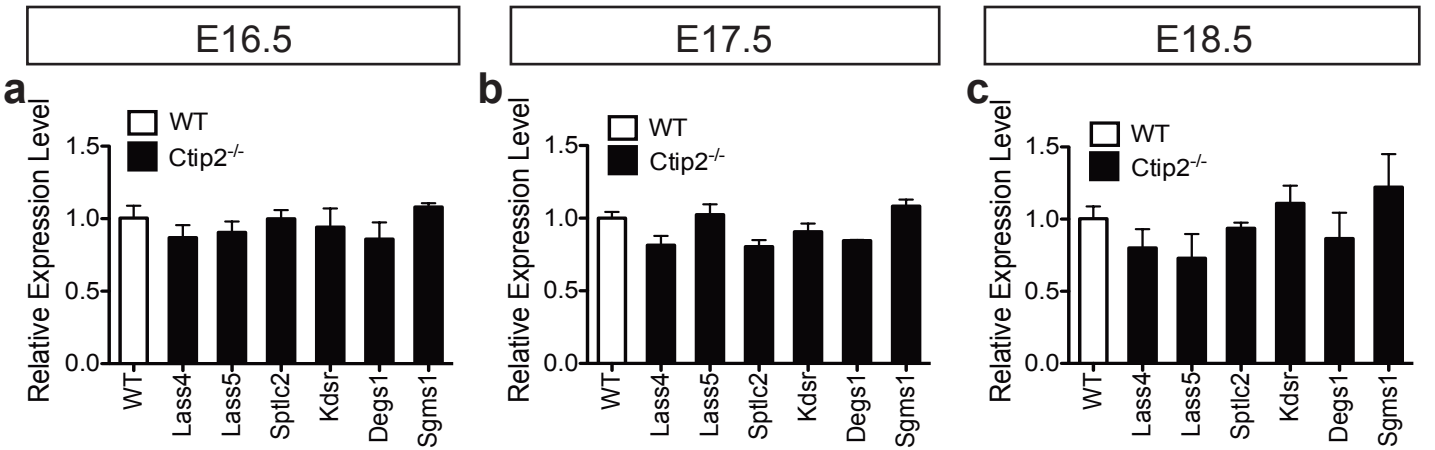

Figure S3, Wang et al., 2012

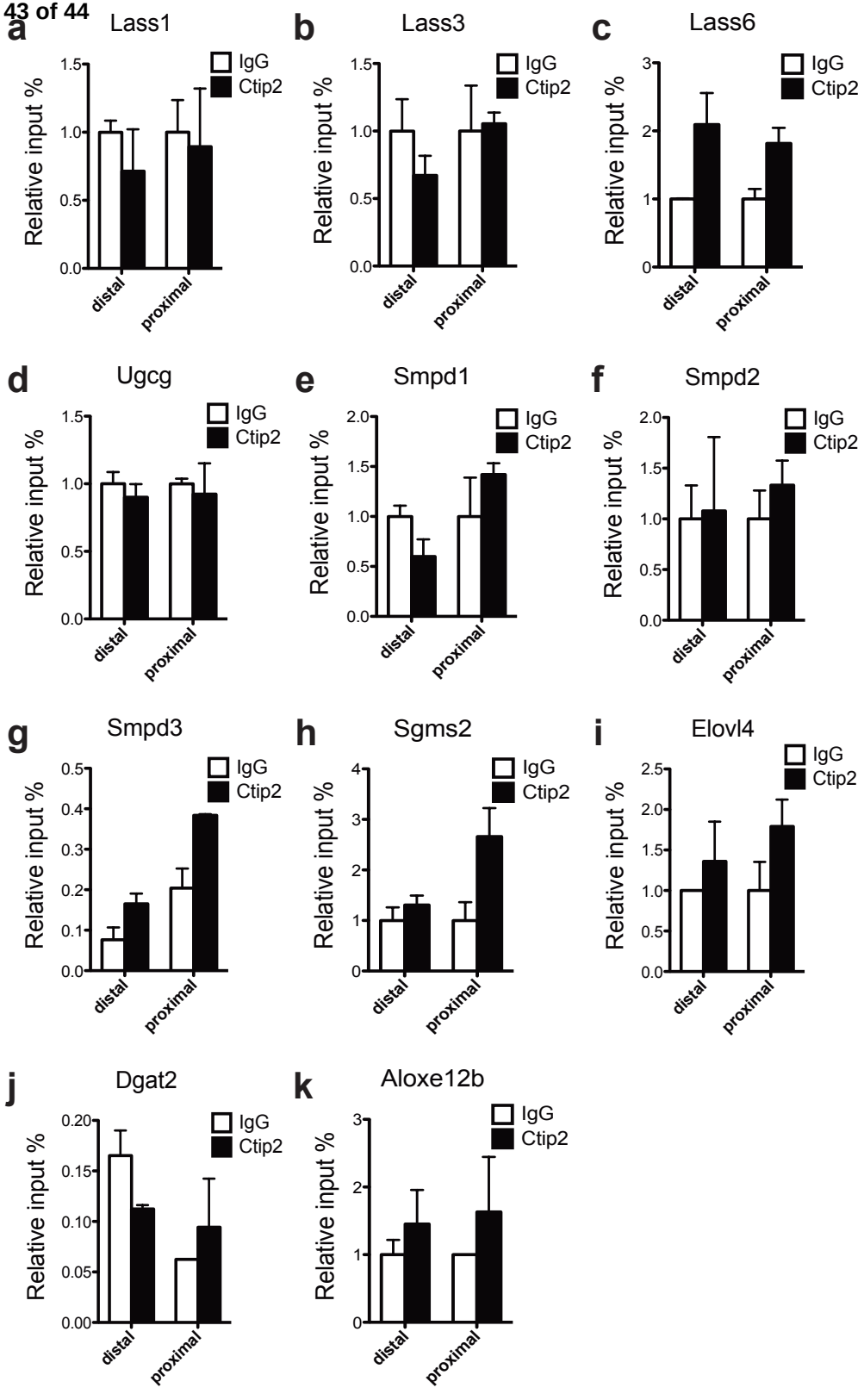

Figure S4, Wang et al., 2012

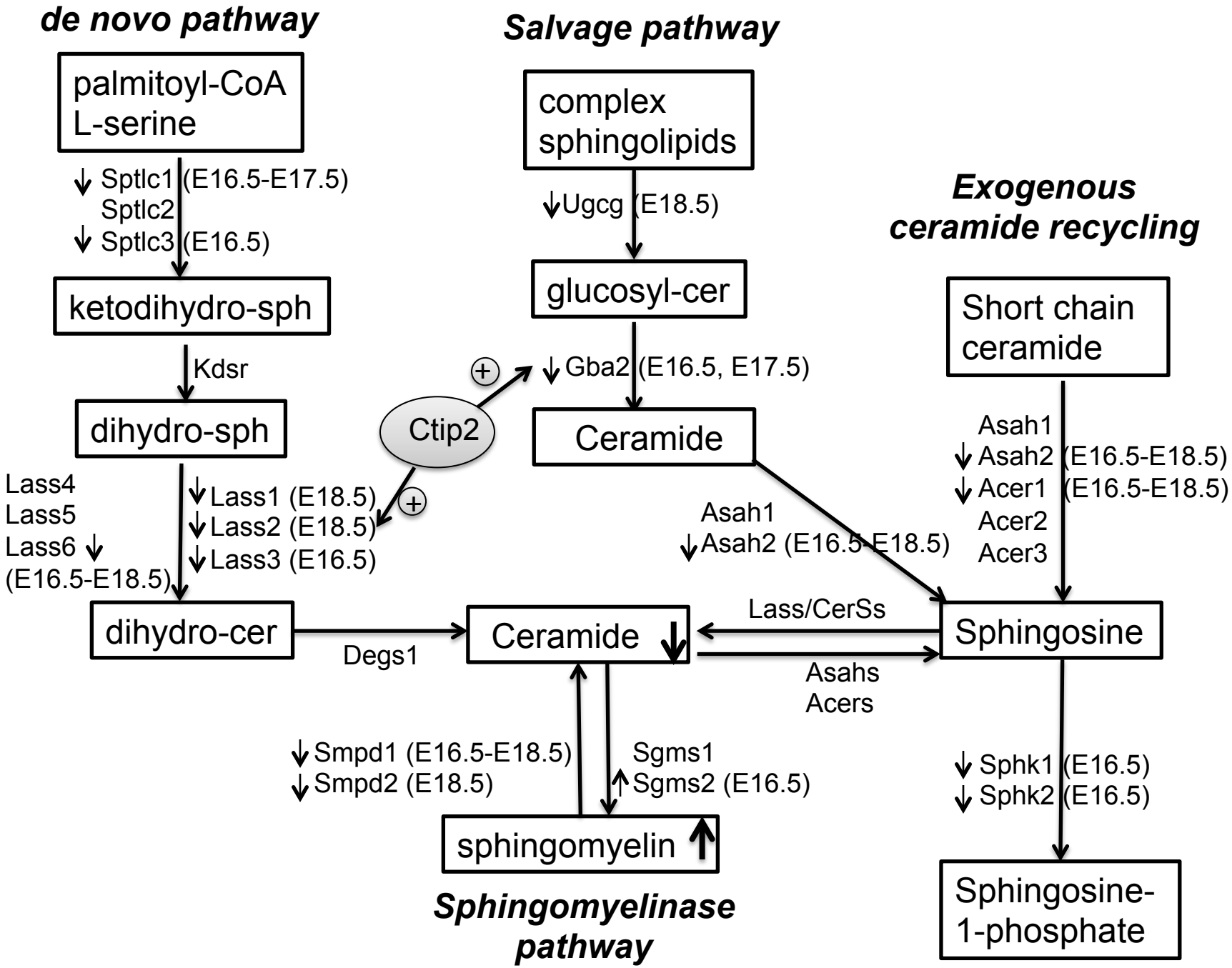

Figure S5, Wang et al., 2012